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MICROBIOLOGICAL TEST RESULTS OF THE ENVIRONMENTAL CONTROL AND LIFE SUPPORT SYSTEMS VAPORS COMPRESSION DISTILLATION SUBSYSTEM RECYLE TANK COMPONENTS FOLLOWING VARIOUS PRETREATMENT PROTOCOLS

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Microbiological samples were collected from the recycle tank of the vapor compression distillation (VCD) subsystem of the water recovery test at NASA Marshall Space Flight Center following a 68-day run. The recycle tank collects rejected urine brine that has been pretreated with a commercially available oxidant (Oxone) and sulfuric acid and pumps it back to the processing component of the VCD. Samples collected included a water sample and two swab samples, one from the particulate filter surface and a second from material floating on the surface of the water. No bacteria were recovered from the water sample. Both swab samples contained a spore-forming bacterium, *Bacillus insolitus*. A filamentous fungus was isolated from the floating material.

Approximately 1 month after the pretreatment chemicals were changed to sodium hypochlorite and sulfuric acid, a swab of the particulate filter was again analyzed for microbial content. One fungus was isolated, and spore-forming bacteria were observed. These results indicate the inability of these pretreatments to inhibit surface attachment. The implications of the presence of these organisms are discussed.

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INTRODUCTION

The Vapor Compression Distillation (VCD) subsystem, used in the Water Recovery Test being performed at NASA/MSFC in support of the Environmental Control and Life Support Systems for Space Station Freedom, processes urine for crew reuse. Prior to being pumped into the subsystem, the raw urine is pretreated, using a commercially available oxidant (Oxone) and sulfuric acid (to acidify the solution). During processing, a recycle tank within the VCD collects rejected pretreated urine (brine) from the VCD, draws it through a particulate filter (45 um), and pumps it back to the processing component of the VCD. This cycle continues until the brine concentration in the recycle tank reaches a predetermined percentage.

Microbiological samples were collected from the recycle tank following a 68-day run of the subsystem in which a brine concentration of approximately 25 percent was reached. Samples collected included a water sample, and two swab samples, one from the particulate filter surface and a second from material floating on the surface water. The pretreatment chemicals were subsequently changed to sodium hypochlorite and sulfuric acid and the subsystem was restarted. Approximately 1 month later, a swab of the particulate filter was collected by the EL branch and given to the laboratory for microbial analysis. This report describes the results of all analyses performed.

MATERIALS AND METHODS

Viable microbial populations from each sample were determined using the spread plate technique. Water samples were collected by pipetting a 10 ml volume of liquid from the recycle tank into a sterile polypropylene test tube. A 0.5 ml aliquot was then placed on R2A agar and spread evenly over the surface using a sterile glass rod. Swab samples were transferred to sterile tubes containing 1 ml of sterile phosphate buffered saline (PBS), vortexed, and a 0.5 ml aliquot was transferred to R2A agar and plated as for the water sample. All samples were incubated for up to 8 days at 28^{o}C.

Total microbial populations from each sample were determined using the epifluorescent microscopy technique. A 1 ml volume of the water sample was resuspended in 10 ml PBS and filtered across a 0.22 um black polycarbonate filter. Approximately 2 ml of acridine orange (0.01%) was added to the filter surface and then removed after 2 minutes. The filter was then rinsed with sterile PBS. A portion of material from each swab sample was placed on a 0.22 um black polycarbonate filter, teased apart, and stained as above. Samples were examined under a Nikon light microscope adapted for epifluorescent microscopy using 40X and 100X objectives with a 10X eyepiece.

Some samples were examined for the presence of bacterial spores using the Wirtz-Conklin spore stain procedure. Prepared slides were observed at 1000X under a Nikon light microscope.

RESULTS

Water

No microbial growth was found in the water sample plated on R2A agar. Epifluorescent microscopy of this sample indicated the presence of bacteria, although levels were below the detection limit of this method (9.54E+04 cells/100ml).

Swab of Material on Liquid Surface

One bacterium, Bacillus insolitus, was isolated on R2A agar from swab samples of material floating on the surface of the recycle tank following oxone pretreatment. A white, filamentous fungus was also isolated from this sample. Epifluorescent microscopy of this sample revealed a long filamentous mold (Figure 1), presumably the same as recovered on R2A agar. Examination of the sample under a light microscope revealed bacteria in various stages of spore development (Figure 2).

Filter Surface Swabs

Two bacterial species were isolated on R2A agar, Clavibacter michiganense and Bacillus insolitus, following the oxone pretreatment. No fungi were isolated on R2A agar. The filter surface produced a high background fluorescence after staining with acridine orange, preventing detailed observation using epifluorescent microscopy. Bacteria in various stages of spore development were again observed (Figure 3). No bacteria were isolated from the filter surface on R2A agar following the sodium hypochlorite pretreatment, although spore-forming bacteria were observed under the light microscope. A single fungal type was recovered from this sample on R2A. This mold was distinct from the one recovered from the swab of material on the liquid surface following oxone pretreatment in terms of both colonial morphology (green) and cellular morphology (Figure 4).

DISCUSSION

Bacillus spp. and fungi, which have been observed in the VCD before, are selected for by the low pH of the subsystem. Fungi, which grow readily at low pH, and bacilli, which form resistant spores (evident in this study), are therefore expected. Virtually all species of Bacillus are considered nonpathogenic but their presence in water supplies imparts an unpleasant odor and taste. These bacteria have been isolated from both raw and

product water sources during the WRT (1), but levels have not been high enough to be considered a nuisance. The presence of fungi can pose problems both in terms of health risk and material integrity (due to release of corrosive products). The density of these microorganisms in product water from the WRT appears to be low, indicating adequate removal during processing and, therefore, minimal health risks.

It is more difficult to assess the effect of these microorganisms, particularly fungi, in terms of corrosion of materials. This is especially true in the VCD and surrounding piping that is also exposed to a harsh chemical pretreatment process. Degradation of materials should be carefully followed in systems such as this and if found, examined for both biological and chemical corrosion effects. It is conceivable that the chemical constituent may initiate the corrosion process and the fungi, which require time for establishment, may then accelerate the process. An additional consequence of their presence is the premature failure of system components, such as filtration units. On two separate occasions during this study, gelatinous material was found floating in the VCD recycle tank. Epifluorescent analysis of this material revealed long, filamentous fungi in association with bacteria and other particulate material. The adherence of these materials to the filter surface can dramatically reduce flow through the filter, thereby reducing the operating efficiency of the subsystem.

SUMMARY

The presence of fungi and bacteria in the recycle tank of the vapor compression distillation subsystem indicated that the pretreatment processes employed were ineffective in elimination of all microorganisms. Their presence only in association with surfaces such as the filter and floating debris, may represent a means similar to bacterial survival in biofilms whereby they can survive the pretreatment process. In addition, the isolated microorganisms are uniquely adaptable to this environment. Bacillus sp., the primary bacterial isolate, is capable of forming spores in response to harsh environmental conditions. As these conditions are removed, the spores revert back to a reproductive state.

FUTURE WORK

In cooperation with the Corrosion Research Branch (EH24) and Dr. Dan Walsh of California Polytechnic State University, studies have begun on the effects of various urine pretreatment protocols in terms of microbial persistence and material degradation of various metals, including those currently proposed for the Water Recovery System (WRS) for Space Station

Freedom. Results of these studies will be presented in future NASA Technical Memorandums.

REFERENCES

1. Roman, M.C., and S.A. Minton. 1992. Microbiology Report for Phase III Stage A Water Recovery Test. NASA Technical Memorandum # 103584.

APPROVAL

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The information in this report has been reviewed for technical content. Review of any information concerning Department of Defense or nuclear energy activities or programs has been made by the MSFC Security Classification Officer. This report, in its entirety, has been determined to be unclassified.

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